Lab Notebook: August 3, 2016
Transformation of pSB1C3, Trial 1

Group: Quantitative Bio

We are transforming iGEM's standardized pSB1C3 plasmid into competent DH5-alpha *E. coli* cells for amplification. The selective marker coded in the plasmid is chloramphenicol resistance. It also codes for the mRFP1 gene which produces a red fluorescent colour when expressed. Once we are prepared to clone the Cld-sp and Cld+sp genes, they will be inserted between the XbaI and PstI restriction sites, effectively replacing mRFP1.

Procedure:

Transformation of pSB1C3:

1. Add 1 µL of pSB1C3 to a tube of DH5-alpha *E. coli* cells.
2. Place tube in an ice bucket and let it sit for 30 minutes.
3. Heat shock the tube in a heating bath set at 42°C for 90 seconds.
4. Transfer tube to the ice bucket and let it sit for 5 minutes.
5. Add 1 mL of LB Broth to the tube.

6. Incubate tube for approximately 1 hour at 37°C.
   In: 11:43 am *Out: 12:43 pm*

7. Pipette 5 mL of LB Broth to a culture tube.
8. Add 15 µL of stock chloramphenicol antibiotic solution to the culture tube.
9. Pipette 100 µL of transformed cells.
10. Incubate the culture tube in the shaker at 37°C overnight.

Summary:

- To amplify pSB1C3, we transformed the plasmid to DH5-alpha *E. coli* cells.

Lab Notebook: August 4, 2016
Transformation of pSB1C3, Trial 2

Group: Quantitative Bio

There is no growth in the culture tube as the cells likely did not have the plasmid coding for the chloramphenicol resistance, therefore the transformation must be repeated. We increased the volume of added recombinant plasmid from 1 µL to 10 µL and decreased the volume of added chloramphenicol antibiotic stock solution from 15 µL to 5 µL. We also added 200 µL of transformed *E. coli* cells instead of 100 µL.

Procedure:

Transformation of pSB1C3:

1. Add 10 µL of pSB1C3 to a tube of DH5-alpha *E. coli* cells.
2. Place tube in an ice bucket and let it sit for 30 minutes.
3. Heat shock the tube in a heating bath set at 42°C for 90 seconds.
4. Transfer tube to the ice bucket and let it sit for 5 minutes.
5. Add 1 mL of LB Broth to the tube.

6. Incubate tube for approximately 1 hour at 37°C.
   In: 11:43 am *Out: 12:43 pm*

7. Pipette 5 mL of LB Broth to a culture tube.
8. Add 5 µL of stock chloramphenicol antibiotic solution to the culture tube.
9. Pipette 200 µL of transformed cells.
10. Incubate the culture tube in the shaker at 37°C overnight.

Summary:

- There is no growth in the culture tubes.
- Transformation of pSB1C3 is repeated.
- We increased the volume of pSB1C3 plasmid from 1 µL to 10 µL.
- We increased the volume of added transformed *E. coli* cells from 100 µL to 200 µL.
- We decreased the volume added chloramphenicol antibiotic stock solution from 15 µL to 5 µL.

Lab Notebook: August 4, 2016
Activated Charcoal Experiment

Group: Perchlorate Team

The purpose of this experiment is to test activated charcoal's maximum ability to bind perchlorate ions to it.

Observation:

Sample	Conductivity (units?)	Description
Activated charcoal tube with distilled water.	446	Initial pouring of distilled water in the tube. It was shook and centrifuged and then the conductivity was measured.
Activated charcoal tube with distilled water.	220	Second time filling the tube with distilled water and repeating the process.
Activated charcoal tube with distilled water.	127	Third time filling the tube with distilled water and repeating the process.
Activated charcoal tube with distilled water.	23	Fourth time filling the tube with distilled water and repeating the process. Conductivity is low enough that further washing is not needed.
Sodium perchlorate solution	489 +- 0.2	In an erlenmeyer flask, a 200 mL solution containing 2.6 g of sodium perchlorate was made and the conductivity was measured.
Sodium perchlorate solution in activated charcoal tube	439	20 mL of the perchlorate solution was poured into the activated charcoal tube.

Dilution

- Initial measurement of activated charcoal: 20 g
- Measurement of activated charcoal after washing with distilled water: 26 g
- 6 g of distilled water is in the tube and in order to pour into the tube a 1% perchlorate solution, a solution of 2.6 g of sodium perchlorate was dissolved in 200 mL solution (1.3% concentrated solution). 20 mL of that solution would be poured into the tube. That means that 0.26 g of sodium perchlorate would be poured into the tube and the tube would have 26 mL of solution (20 mL + 6 mL). 0.26 g of sodium perchlorate in 26 mL solution is 1% perchlorate solution.

Activated charcoal binding capacity

- The difference in conductivity from the perchlorate solution and the perchlorate solution in activated charcoal determines the binding capacity of activated charcoal. A difference from 489 to 439 was observed.

Summary:

- We measured and placed 20 g of activated charcoal in a 50 mL tube.
- To increase the capacity of the activated charcoal binding to perchlorate and determine its maximum capacity, the activated charcoal was to be rinsed of unnecessary compounds and elements. This was done by filling the 50 mL tube to the top with distilled water and centrifuging the tube in order to force the unnecessary compounds out of the activated charcoal. The conductivity of the supernatant was measured and afterwards removed.
- Each time after we added distilled water to the tube and centrifuged the tube, we measured the conductivity of the solution. The more conductive the solution was, the more ions were present in the solution.
- We repeated this process until the conductivity decreased to a relatively low amount that only trace amounts of unnecessary compounds were left in the activated charcoal (hard to remove).
- The activated charcoal (insoluble material) remained at the bottom of the tube.
- After the rinsing process of the activated charcoal, we poured out the supernatant and remeasured the mass of the activated charcoal.
- Accounting for the increase in mass due to distilled water contained in the activated charcoal, we had to make a 1.3% concentrated sodium perchlorate solution. This was done because the distilled water contained in the activated solution would dilute the concentration of sodium perchlorate solution added to a 1% concentrated solution.
- A measurement of the conductivity of the sodium perchlorate solution was made.
- 20 mL of the sodium perchlorate solution was added to the 50 mL tube containing the activated charcoal.
- The conductivity of the sodium perchlorate solution in the tube of the activated charcoal was measured and compared to the conductivity of the sodium perchlorate solution.

Lab Notebook: August 5, 2016
Activated Charcoal Experiment, Continued

Group: Perchlorate Team

Summary

- We left the 50 mL tube filled with 20 g of activated charcoal and a 1% perchlorate solution (2.6 g per 26 mL) overnight rotating to see if the activated charcoal's ability to bind perchlorate is affected by time.

Lab Notebook: August 8, 2016
Transforming Cld+sp and Cld-sp Plasmids and Plating

Group: Quantitative Bio

There is no growth in the culture tube as the cells likely did not have the plasmid coding for the chloramphenicol resistance. BioBricks Team is put on hold, and QuantBio team rises from the dead. We are using the extracted plasmids with the Cld+sp and Cld-sp genes. We picked three samples with the highest pDNA concentrations in each group: Cld+sp 2, 4 and 6, and Cld-sp 2, 3 and 4.

Procedure:

Transformation

1. Label three microcentrifuge tubes from 1 to 3 with a subtitle of “Cld+”. Label three other microcentrifuge tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 80 µL of competent DH5-alpha *E. coli* cells into every microcentrifuge tube.
3. Pipette 1 µL of cloned plasmid into the appropriate tube.
4. Place tubes in an ice bucket and let it sit for 30 minutes.
5. Heat shock the tubes in a heating bath set at 42°C for 90 seconds.
6. Transfer tubes to the ice bucket and let it sit for 5 minutes.
7. Add 1 mL of LB Broth to every tube.

8. Incubate tubes for approximately 1 hour at 37°C.
   In: 11:30 am *Out: 12:30 pm*

Preparation of Agar Kanamycin Plates

Plating

1. Label three plates from 1 to 3 with a subtitle of “Cld+”. Label three other plates tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 200 µL of transformed *E. coli* cells from each of the tubes into the appropriate plates.
3. Gently spread the liquid over the entire area of the plates using a spreader.
4. Place all plates under the fume hood with lids off until they are dry.
5. Flip plates (lid on the bottom) and incubate at 37°C overnight.

Summary:

- There is no growth in the culture tubes incubated overnight.
- Transformed and plated cells with our recombinant plasmid.

Lab Notebook: August 9, 2016
Inoculation of Cld+sp and Cld-sp Colonies

Group: Quantitative Bio

Evident from the low number of colonies in the plates, our transformation efficiency is quite low. However, there are relatively more colonies in Cld-sp plates than in Cld+sp plates. A few purples colonies were also spotted in the plates. We might have to change our transformation protocols to increase the efficiency. We are inoculating the colonies from the plates into the culture tubes.

Procedure:

1. Label three culture tubes from 1 to 3 with a subtitle of “Cld+”. Label three other culture tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 100 µL of 1000x kanamycin stock solution into 100 mL of LB Broth.
3. Pipette 5 mL of LB Broth with kanamycin antibiotic into every tube.
4. Using the needle end of the inoculation loop, scrape a white colony from each plate and vigorously mix into the appropriate tube.
5. Place all tubes at an angle in the shaker and incubate at 37°C overnight.

Summary:

- Only a small number of colonies thrived in the plates, with more colonies thriving in the Cld-sp plates than in the Cld+sp plates.
- A few purple colonies are spotted in the plates.
- Inoculation with white colonies to prepare overnight cultures.

Lab Notebook: August 10, 2016
Inoculating Terrific Broth with Overnight Cultures

Group: Quantitative Bio

We are inoculating the overnight cultures to terrific broth with kanamycin. Incubating the cultures in Terrific Broth media should theoretically aid them in growing to their mid-log phase (A600 0.6-0.8) at a faster rate compared to when they are incubated with LB Broth media. Once they reach this mid-log phase, we induce the cultures with IPTG (Isopropyl β-D-1-thiogalactopyranoside). The lac repressor binds to the lac operators flanking the T5 promoter. IPTG binds to the lac repressor, effectively releasing its bind on the lac operator, and consequently turning on the gene (https://www.dna20.com/eCommerce/catalog/datasheet/94). Since chlorite dismutase has a heme component in its protein structure, we are also adding aminolevulinic acid (a precursor for heme) and ferric sulfate to aid in the production of heme. All tubes have relatively consistent bacterial density, except for the Cld+sp 2 and 3 tubes which contain comparatively less *E. coli*. We will pipette 10 µL from these tubes to inoculate the Terrific Broth media, while we will use 5 µL for the rest of the tubes.

Procedure:

1. Label three culture tubes from 1 to 3 with a subtitle of “Cld+”. Label three other culture tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 50 µL of 1000x kanamycin stock solution into 50 mL of Terrific Broth.
3. Pipette 5 mL of Terrific Broth with kanamycin antibiotic into every tube.
4. Pipette 5 µL of each of the overnight culture into the appropriate tube, except for the Cld+sp 2 and 3 tubes. Pipette 10 µL for the Cld+sp 2 and 3 tubes.
5. Place all tubes at an angle in the shaker and incubate at 37°C for approximately 5 hours or until the culture’s mid-log phase.

Result:
After 5 hours of incubation, the culture tubes were still clear. The cells were not in the mid-log phase. It has been decided to use LB Broth instead of Terrific Broth for incubation, as it seems that bacterial cells grow at a faster rate in LB.

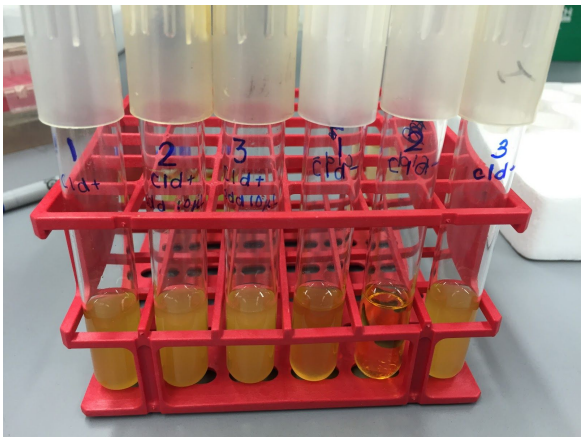
Summary:

- The bacterial density in the Cld+sp 2 and 3 overnight cultures were relatively less than the others.
- Inoculation of Terrific Broth with overnight cultures.
- The cultures with Terrific Broth media did not grow to mid-log phase in the expected time frame.

Lab Notebook: August 11, 2016
Inoculation and Incubation of Cld-sp and Cld+sp and Gene Expression

Group: Quantitative Bio

All of the Terrific Broth overnight cultures thrived, except for the Cld-sp 2 tube where no bacterial growth has been observed. We will no longer be using Terrific Broth for incubation and instead return to using LB Broth media, as the cultures reach their mid-log phase in approximately 5 hours. We are also inoculating every tube with two colonies.



Procedure:

Inoculation and Incubation

1. Label three culture tubes from 1 to 3 with a subtitle of “Cld+”. Label three other culture tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 50 µL of 1000x kanamycin stock solution into 50 mL of LB Broth.
3. Pipette 5 mL of LB Broth with kanamycin antibiotic into every tube.
4. Using the needle end of the inoculation loop, scrape a white colony from each plate and vigorously mix into the appropriate tube. Do this step twice to inoculate all tubes with two colonies.
5. Place all tubes at an angle in the shaker and incubate at 37°C for approximately 5 hours or until the culture’s mid-log phase.

Induction with IPTG, and Addition of Aminolevulinic Acid and Ferric Sulfate

1. Pipette 50 µL of IPTG into every tube.
2. Pipette 100 µL of 0.1M stock solution of aminolevulinic acid into every tube.
3. Pipette 500 µL of 20 mM stock solution of ferric sulfate into every tube.
4. Incubate all tubes in the shaker at 37°C overnight.

Summary:

- We incubated the cells in LB Broth media instead of Terrific Broth.
- At the mid-log phase, we added IPTG, aminolevulinic acid and ferric sulfate and incubated the culture overnight.

Lab Notebook: August 12, 2016
Oxygen Production

Group: Quantitative Bio

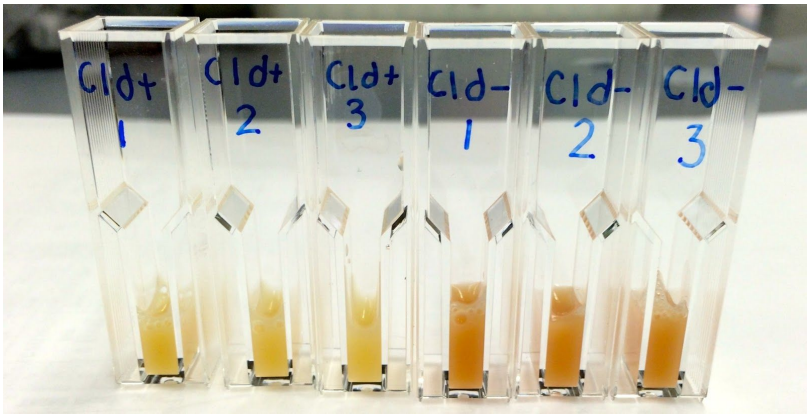
Cultures in the Cld-sp tubes have a darker hue than the Cld+sp cultures. This is likely due to more of the Cld-sp enzymes incorporating heme to their structure, resulting to a darker brownish colour. The signal peptide in Cld+sp transports the enzyme across the inner membrane to the periplasmic space, where we expected to have greatest concentration of chlorite ions. However, the inner membrane is not permeable to heme, and it therefore remains in the cytoplasm unable to bind to the greater concentration of chlorite dismutase in the periplasmic space. Cld-sp does not include the signal peptide and therefore remains in the cytoplasm, effectively integrating heme to its structure. In this experiment, we added a solution of sodium chlorite to resuspended pellets of our overnight cultures in order to test for the expression of the chlorite dismutase gene. Bubbles indicate that we have successfully expressed chlorite dismutase in *Escherichia Coli*.

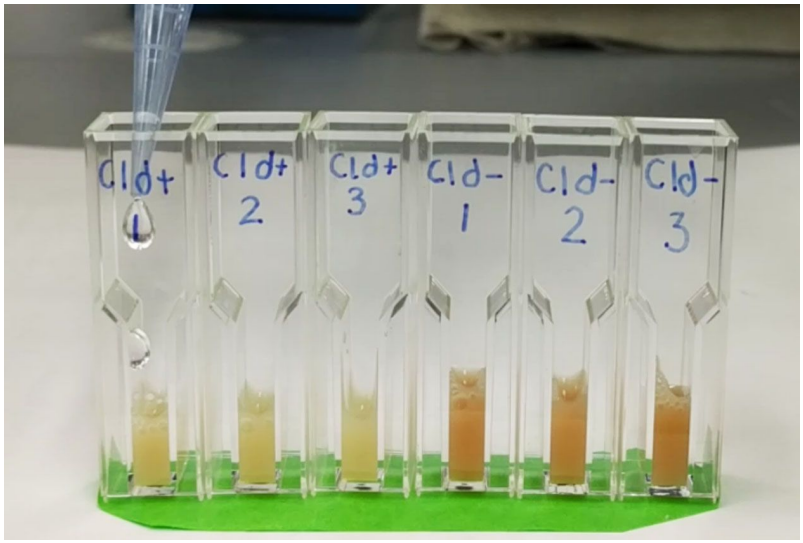
Procedure:

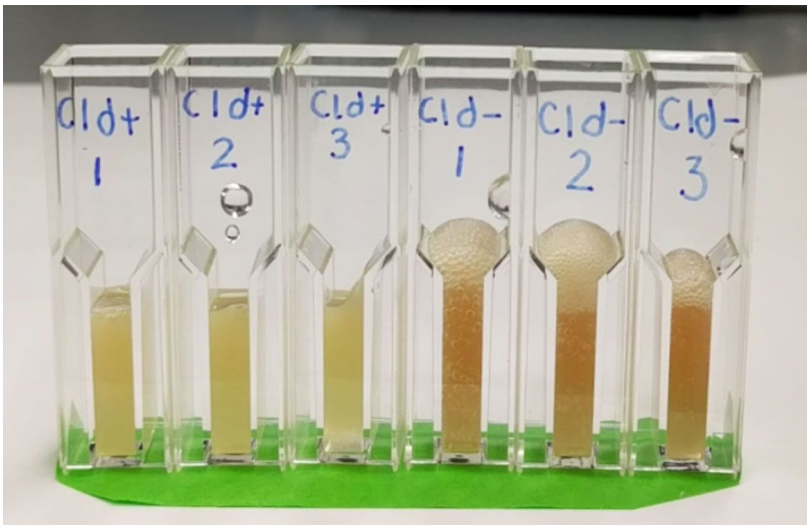
1. Label three microcentrifuge tubes from 1 to 3 with a subtitle of “Cld+”. Label three other microcentrifuge tubes from 1 to 3 with a subtitle of “Cld-”.
2. Pipette 1 mL of every overnight culture from the culture tubes into their corresponding microcentrifuge tubes and centrifuge at 13 000 rpm for 1 minute. Decant the supernatant. Repeat this step until all 5 mL of culture have been centrifuged. If needed, pipette out the remaining liquid.
3. Pipette 300 µL of Buffer EB into every tube and resuspend the pellets by vortexing.
4. Dissolve 0.1094 g of sodium chlorite into 10 mL of Milli-Q water.
5. Label three cuvette tubes from 1 to 3 with a subtitle of “Cld+”. Label three other cuvette tubes from 1 to 3 with a subtitle of “Cld-”.
6. Pipette 400 µL of each of the resuspended pellets into their corresponding cuvette tube.
7. Pipette 400 µL of sodium chlorite solution to every tube.
8. Quickly cover the tubes with a sheet of parafilm.

Observations:

- There seems to be more pellet volume in Cld-sp tubes than in Cld+sp tubes. It is likely that there is more bacterial growth in Cld-sp tubes, because no extra resources need to be consumed for the expression of a signal peptide.
- More bubbles are produced in tubes containing Cld-sp than in tubes containing Cld+sp.







Summary:

- Cld-sp overnight cultures are darker than Cld+sp overnight cultures. This is likely due to Cld-sp incorporating heme more effectively.
- Added sodium chlorite to resuspended pellets of overnight cultures.
- There is more pellet volume in Cld-sp tubes than in Cld+sp tubes. This is likely due to greater bacterial density in Cld-sp tubes.
- More oxygen bubbles are produced in tubes containing Cld-sp than in tubes containing Cld+sp.

Lab Notebook: August 15, 2016
Preparation of an Overnight Culture of Cld-sp 3

Group: Quantitative Bio

We are now scaling our previous successful expression of chlorite dismutase from using 5 mL of culture to 250 mL of culture. We are also incorporating an oxygen monitor to our experiment to calculate the volume of oxygen produced. We will stretch a male contraceptive device over the lip of an erlenmeyer flask. Its inflated figure allow for simple volume measurements and it is more sensitive to changes in pressure as compared to an average balloon, thus making it suitable as an oxygen monitor device. We are no longer focusing on Cld+sp, as Cld-sp exhibited greater efficiency in producing oxygen. For this experiment, we only picked a colony from the Cld-sp 3 kanamycin plate, as it is the plate without purple colonies and has more colonies when compared to other Cld-sp plates.

Procedure:

1. Label one culture tube as “Cld-”. Label another culture tube as “control”.
2. Pipette 5 mL of LB Broth into each culture tube.
3. Pipette 5 µL of 1000x kanamycin stock solution into the “Cld-” culture tube.
4. Using the needle end of the inoculation loop, scrape a white colony from the Cld-sp 3 kanamycin plate and vigorously mix into the “Cld-” tube.
5. Pipette 15 µL of competent DH5-alpha *E. coli* cells into the “control” tube.
6. Place all tubes at an angle in the shaker and incubate at 37°C overnight

Summary:

- We prepared an overnight culture from a colony on the Cld-sp 3 kanamycin plate.
- A culture of DH5-alpha *E. coli* cells without any plasmid stood as our control group.

Lab Notebook: August 16, 2016
Inoculation with Overnight Cultures and Gene Expression

Group: Quantitative Bio

The two tubes have no discernible difference. The bacterial density in both tubes are nearly the same. We are inoculating erlenmeyer flasks containing 250 mL of LB Broth media with overnight cultures. Once they reach their mid-log phase, we are inducing them with IPTG, and adding aminolevulinic acid and ferric sulfate.

Procedure:

Inoculation and Incubation

1. Label one erlenmeyer flask as “Cld-”. Label another erlenmeyer flask as “control”.
2. Pipette 250 mL of LB Broth into each erlenmeyer flask.
3. Pipette 250 µL of 1000x kanamycin stock solution into the “Cld-” erlenmeyer flask.
4. Pipette 250 µL of each of the overnight culture into their corresponding erlenmeyer flask.
5. Place both flasks in the shaker and incubate at 37°C for approximately 5 hours or until the culture’s mid-log phase.

Induction with IPTG, and Addition of Aminolevulinic Acid and Ferric Sulfate

1. Pipette 2.5 mL of IPTG into every flask.
2. Pipette 5 mL of 0.1M stock solution of aminolevulinic acid into every flask.
3. Pipette 25 mL of 20 mM stock solution of ferric sulfate into every flask.
4. Incubate both flasks in the shaker at 37°C overnight.

Summary:

- We inoculated 250 mL of LB Broth with overnight cultures.
- When the cultures reached their mid-log phase, we added IPTG, aminolevulinic acid and ferric sulfate, and incubated the cultures overnight.

Lab Notebook: August 17, 2016
Preparation of Overnight Cultures of Cld-sp 1, 2 and 3

Group: Quantitative Bio

The culture in the "Control" erlenmeyer flask has a darker orange hue than the culture in the "Cld-sp" erlenmeyer flask. The culture in the "Cld-sp" erlenmeyer flask does not have the expected brownish colour. It is likely that the Cld-sp gene block was not expressed properly. To test for the presence of the chlorite dismutase enzyme, we pipetted 2 mL of culture from the "Cld-sp" erlenmeyer flask into a culture tube. We then added a small mass of solid sodium chlorite to the culture. When no bubbles were observed, we decided to repeat the experiment and scale it down from 250 mL of culture to 50 mL of culture. We are also preparing cultures for all three Cld-sp kanamycin plates.

Procedure:

1. Label three culture tubes from 1 to 3 with a subtitle of "Cld-". Label another culture tube as "control".
2. Pipette 30 µL of 1000x kanamycin stock solution into 30 mL of LB Broth.
3. Pipette 5 mL of LB Broth with kanamycin antibiotic into each of the "Cld-" culture tube.
4. Pipette 5 mL of fresh LB Broth into the "Control" culture tube.
5. Using the needle end of the inoculation loop, scrape a white colony from each of the Cld-sp kanamycin plates and vigorously mix into their corresponding culture tube.
6. Inoculate the "Control" culture tube with 2 µL of overnight culture from the "Control" erlenmeyer flask.
7. Place all tubes at an angle in the shaker and incubate at 37°C overnight

Summary:

- The cells in the "Cld-sp" erlenmeyer flask did not express the g-block.
- We plan to scale down the experiment from using 250 mL of culture to 50 mL of culture.
- We prepared three overnight cultures from colonies in the Cld-sp kanamycin plates.
- To inoculate the "Control" culture tube, we pipetted 2 µL of overnight culture from the "Control" erlenmeyer flask.

Lab Notebook: August 18, 2016
Inoculation with Overnight Cultures and Gene Expression

Group: Quantitative Bio

The tubes have no discernible difference. The bacterial density in all tubes are nearly the same. We are inoculating erlenmeyer flasks containing 50 mL of LB Broth media with overnight cultures. Once they reach their mid-log phase, we are inducing them with IPTG, and adding aminolevulinic acid and ferric sulfate.

Procedure:

Inoculation and Incubation

1. Label three erlenmeyer flasks from 1 to 3 with a subtitle of “Cld-”. Label another erlenmeyer flask as “control”.
2. Add 50 mL of LB Broth into each erlenmeyer flask.
3. Pipette 50 µL of 1000x kanamycin stock solution into each of the “Cld-” erlenmeyer flasks.
4. Pipette 20 µL of each of the overnight culture into their corresponding erlenmeyer flask.
5. Place all flasks in the shaker and incubate at 37°C for approximately 5 hours or until the culture’s mid-log phase.

Induction with IPTG, and Addition of Aminolevulinic Acid and Ferric Sulfate

1. Pipette 500 µL of IPTG into every flask.
2. Pipette 1 mL of aminolevulinic acid into every flask.
3. Pipette 5 mL of ferric sulfate into every flask.
4. Incubate both flasks in the shaker at 37°C overnight.

Summary:

- We inoculated 50 mL of LB Broth with overnight cultures.
- When the cultures reached their mid-log phase, we added IPTG, aminolevulinic acid and ferric sulfate, and incubated the cultures overnight.

Lab Notebook: August 19, 2016
Oxygen Production Upscaled

Group: Quantitative Bio

The erlenmeyer flasks containing the culture with Cld-sp had a darker orange hue than the control, most likely due to the Cld-sp incorporating the heme.

Summary:

- We added 0.1 M of solid sodium chlorite to one of the flasks, which contained 50 mL of culture, measuring out to be 0.9 g of sodium chlorite.
- We added a stir bar to the flask and placed it onto a magnetic stirrer to fully incorporate the sodium chlorite into the culture.
- A male contraceptive was used to trap and measure the oxygen production.
- Due to the low output of oxygen, we decided to increase the amount of sodium chlorite we add to the cultures and combine the remaining two cultures.
- We repeated the experiment, this time adding 1.8 g of solid sodium chlorite, which produced a greater volume of oxygen.
- The experiment was then conducted on the control flask, which did not produce any discernable results.
- To find the wet and dry weight of the culture, we transferred it into a centrifuge tube and centrifuged for 10 minutes, until a pellet was formed.
- We added 500 uL of EB buffer to resuspend the pellet and transferred into an eppendorf tube, which we centrifuged again for 1 minute.
- The wet weight measured out to be 0.5 g and the dry weight was 0.1 g.